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GENETIC VARIATION IN *PINUS PONDEROSA, PURSHIA TRIDENTATA,* AND *FESTUCA IDAHOENSIS*, COMMUNITY-DOMINANT PLANTS OF CALIFORNIA'S YELLOW PINE FOREST

Safiya Samman,[1], Barbara L. Wilson[2] and Valerie D. Hipkins[3]
[1]USDA Forest Service, FHP, AB-2S, P.O. Box 96090,
Washington, D.C. 9009-6090
[2]Department of Botany and Plant Pathology, 2064 Cordley Hall,
Oregon State University, Corvallis, OR 97331
[3]USDA Forest Service-NFGEL, 2375 Fruitridge Road, Camino, CA 95709

Abstract

Genetic diversity of *Pinus ponderosa* Laws., *Purshia tridentata* (Pursh) DC., and *Festuca idahoensis* Elmer at Black's Mountain Experimental Forest in northwest California was evaluated using isozymes. This tree, shrub, and grass are all common, outcrossing, long-lived perennials that dominate their respective layers of the same plant community. Genetic analyses were provided for diploid *Pinus ponderosa* and *Purshia tridentata.* A phenotypic analysis of isozyme band patterns was provided for tetraploid *F. idahoensis* and, for comparison, previous reports of fescue isozyme variation were reanalyzed using this method. *Pinus ponderosa, Purshia tridentata,* and *Festuca idahoensis* were highly genetically variable, with 75% to 92% polymorphic loci. For all three species, more than 90% of the genetic variation occurred within, rather than among, populations.

This study compares genetic diversity in plant species of three life forms, while holding constant habitat, breeding system, and community dominance. The three plant species chosen for study are *Pinus ponderosa* Laws, *Purshia tridentata* (Pursh) DC, and *Festuca idahoensis* Elmer. They represent three life forms, tree, shrub, and grass, respectively. All three are common, widespread, outcrossing, long-lived perennials. All dominate their respective layers in the plant community at the study site. They do have life history differences; *F. idahoensis* is insect pollinated while the other two species are wind pollinated, and *F. idahoensis* is tetraploid while the others are diploid. The three species affect one another in a complex web of competitive and commensal relationships (e.g., Baron et al. 1966; Busse et al. 1996; Hall et al. 1995; vander Wall and vander Wall 1992).

The study site, Black's Mountain Experimental Forest, was established in 1934 in the Lassen National Forest, Lassen County, California. More than 60 y of experimentation and careful record keeping make the 4050-ha forest a uniquely valuable resource for investigating the effects of different timber management practices on eastside pine type forests. In 1993 the Black's Mountain Interdisciplinary Research Program was established to study the effects of forest management on various ecosystem components including vertebrates, insects, soil organisms, and vegetation. This study provides baseline data for a long-term study of effects of silvicultural treatments on genetic biodiversity. Genetic and species biodiversity are elements of a healthy ecosystem. Little is known about the effects of forest management on the genetics of forest plants, although some forestry practices can profoundly influence tree genetics (Adams et al. 1998).

Four plots similar in topography and vegetation were chosen for this study (Table 1). Genetic variation in the three selected species was sampled in 1994 and 1995. Subsequently, three silvicultural treatments (a timber cutting regime, fire, and grazing) were applied to the plots (Table 1). Genetic diversity will be resampled in five to twenty years, to detect any effects from the silvicultural practices initiated in 1995.

Methods

In 1993, plots were chosen for an intensive, multidisciplinary study of the effects of management practices on the entire ecosystem. Midpoints of the four plots were 2.2 to 4.0 km apart. These plots have been treated similarly in the past, and all were grazed lightly until 1996, when some were fenced to exclude cattle (Table 1). All four plots sampled in the genetics study consisted of dry forest dominated by *P. ponderosa,* and all plots were similar (Table 1). Half of each plot was subsequently burned. The presence of small, unburned, long-term control plots in some burned split plots is ignored in this analysis.

Sample collection. Permanent markers were established on a 100-m grid within each plot. In each plot, fifty grid points were randomly selected as

Corresponding and proofs author: Valerie D. Hipkins, USDA Forest Service—NFGEL, 2375 Fruitridge Road, Camino, CA 95709. Phone: 530 642-5067. Fax: 530 642-5093. E-mail: vhipkins@fs.fed.us

TABLE 1. CHARACTERISTICS AND MANAGEMENT PLANS OF THE FOUR BLACK'S MOUNTAIN PLOTS SAMPLED IN THIS STUDY. Pine trees are mostly Ponderosa Pine with some Jeffrey Pine; fir trees are White Fir, *Abies concolor* (Gordon and Glend.) Lindley. Perennial grass cover was predominantly Idaho Fescue cover. Structural vertical diversity is a management practice imposed by thinning two of the plots to reduce structural diversity. Half of each plot was subsequently burned; burned and unburned halves are labeled "B" and "N," respectively, in other tables. Information on plot vegetation from W. W. Oliver (unpublished data).

Plot	Elevation (meters)	Area (hectares)	Live trees/hectare		Idaho Fescue (frequency)	Perennial grasses (foliar cover)	Structural Vertical Diversity	Grazing
			Pine	Fir				
38	523–540	136	440	9	59%	4%	high	no
39	526–543	120	333	0	77%	6%	low	no
41	575–580	108	440	16	22%	2%	high	yes
43	570–576	109	364	170	40%	2%	low	yes

collection sites. All three species were collected at each grid point, if all three were present. If one or more was missing, a replacement sample was collected at a different, ranodmly selected grid point.

Pinus ponderosa: Cones were collected from the tree nearest each selected grid point (207 total individuals). Samples were collected in September 1994.

Purshia tridentata: At each selected grid point, two individuals were flagged and sampled (404 total individuals). The shrub closest to the grid point was the first sampled individual, and the second was the closest shrub on the opposite side of the gridpoint, on a line drawn from the first shrub through the grid point. A leafy shoot was cut from each individual, wrapped in wet paper towels, placed in a plastic bag, and stored on ice in the field. Samples were collected in June and July 1995.

Festuca idahoensis: Near each flagged *P. tridentata* shrub, a fescue individual was sampled (385 total individuals). (If fescue was sparse, the fescue might be as much as 30 meters away from the *P. tridentata.*) If no fescue grew within 30 meters of a sampling point, another point was randomly selected for fescue sampling. From each fescue, a small rooted plug with about 20 leaves was collected, wrapped in wet paper towels, placed in a plastic bag, and stored on ice in the field. Samples were collected in July 1995.

Sample preparation. Samples were prepared using NFGEL standard operating procedures (Anonymous 1995). For *P. ponderosa,* seeds were germinated and the megagametophytes were ground in a 0.2 M phosphate buffer, pH 7.5. The slurry was absorbed onto 3 mm wide wicks prepared from Whatman 3MM chromatography paper, and stored at −70°C. *Purshia tridentata* and *F. idahoensis* leaf tissue was ground in a Tris buffer pH 7.5 (Gottlieb 1981); liquid nitrogen was used to freeze *P. tridentata* tissue before grinding. One hundred fifty microliters of slurry per sample was transferred into each of two microtiter plate wells, and plates were frozen at −70°C. For electrophoresis, the slurry was thawed and absorbed onto wicks.

Electrophoresis. Methods of electrophoresis are outlined in Anon. (1995), and follow the general methodology of Conkle et al. (1982) except that most enzyme stains are modified. The following enzymes were examined: aconitase (ACO), catalase (CAT), diaphorase (DIA), florescent esterase (FEST), fructose-1,6-diphosphate dehydrogenase (FDP), glutamate-oxaloacetate transaminase (GOT), glucose-6-phosphate dehydrogenase (G6PDH), glycerate-2-dehydrogenase (GLYDH), isocitrate dehydrogenase (IDH), leucine aminopeptidase (LAP), malate dehydrogenase (MDH), malic enzyme (ME), phosphoglucomutase (PGM), phosphogluconate dehydrogenase (6PGD), phosphoglucose isomerase (PGI), shikimic acid dehydrogenase (SKD), triosephosphate isomerase (TPI), and uridine diphosphoglucose pyrophosphorylase (UGPP). The enzymes examined, and the buffer systems used to resolve them, varied according to species (Table 2). All enzymes were resolved on 11% starch gels. Enzyme stain recipes for enzymes follow Anonymous (1995) except that GOT was stained using the recipe from Wendel and Weeden (1989). Two people independently scored each gel. When they disagreed, a third person resolved the conflict. For quality control, 10% of the individuals were run and scored twice.

Both *P. ponderosa* and the morphologically similar Jeffrey Pine *P. jeffreyi* Grev. and Balf (Jeffrey Pine) occur in the research plots (Oliver, MS). For a few of the pine samples, alleles of multiple enzymes differed from those seen before in *P. ponderosa* (NFGEL, unpublished data). Such samples were omitted from analysis on the assumption that they were *P. jeffreyi.*

Pinus ponderosa and *P. tridentata* are diploid. Most studied isozymes are known to show Mendelian inheritance in *P. ponderosa* (Linhart et al. 1989; O'Malley et al. 1979), but no such information is available for *P. tridentata.* Genetic interpretations were inferred directly from isozyme phe-

TABLE 2. ISOZYMES EXAMINED FOR *PINUS PONDEROSA*, *PURSHIA TRIDENTATA*, AND *FESTUCA IDAHOENSIS*. LB = a lithium borate electrode buffer (pH 8.3) used with a Tris citrate gel buffer (pH 8.3) (Conkle et al. 1982). SB = a sodium borate electrode buffer (pH 8.0) used with a Tris citrate gel buffer (pH 8.8) (Conkle et al. 1982). MC6 = a morpholine citrate electrode and gel buffer (pH 6.1) (Conkle et al. 1982). MC8 = a morpholine citrate electrode and gel buffer (pH 8.0) (Anon. 1985). na = number of alleles per locus. ne = effective number of alleles per locus (Kimura and Crow 1964). np = number of patterns per locus. For enzymes abbreviations, see text. Numbers in isozyme abbreviations refer to different regions on gels, interpreted as different loci.

	Buffer: LB	na	ne	SB	na	ne	MC6	na	ne	MC8	na	ne
PINUS PONDEROSA												
	ACO	4	2.8	CAT	1	1.0	FEST	7	1.5	DIA	4	2.9
16 enzymes	ADH	2	1.9	GOT1	4	1.1	MDH1	3	1.0	FDP	1	1.0
26 loci	LAP1	3	1.1	GOT2	5	1.1	MDH2	3	1.9	IDH1	3	1.7
	LAP2	4	1.1	GOT3	6	1.3	MDH3	4	1.1	IDH2	5	1.2
	ME	4	1.2	G6PDH	4	1.1				6PGD1	5	2.0
	PGI1	2	1.0	UGPP1	3	1.9				6PGD2	4	1.1
	PGI2	3	1.1	UGPP2	6	3.0						
	PGM1	4	1.9									
	PGM2	3	2.3									
PURSHIA TRIDENTATA		na	ne		na	ne					na	ne
	FEST	1	1.0	CAT	2	1.0				DIA	2	1.0
13 enzymes	LAP	3	2.6	GOT	4	2.6				FDP	3	1.0
16 loci	PGI1	1	1.0	6PGD	4	1.1				IDH	1	1.0
	PGI2	3	1.0	TPI1	2	1.0				MDH1	2	1.1
	PGM	3	2.0	TP12	1	1.0				MDH2	3	1.0
				UGPP	3	1.9						
FESTUCA IDAHOENSIS		np			np						np	
	ME	1		CAT	1					DIA	6	
11 enzymes	PGI	17		GLYDH	3					SKD	4	
treated as 12 loci	PGM	3		GOT1	3							
				GOT2	4							
				6PGD	2							
				TPI	4							
				UGPP	16							

notypes, based on knowledge of the generally conserved enzyme substructure, compartmentalization, and isozyme number in higher plants (Gottlieb 1981, 1982; Weeden and Wendel 1990). Genotypes of *P. ponderosa* were inferred from the segregation patterns of 10 megagametophytes per tree.

Festuca idahoensis has a chromosome number 2n = 28 and is thought to be autotetraploid (Darlington and Wylie 1955). Because of the complicated banding patterns observed, and because of lack of crossing studies to determine the inheritance of bands in this species, we were unable to identify specific alleles and loci for some enzymes, including highly variable MDH, PGI and UGPP. Therefore, a phenotypic instead of genotypic analysis was performed.

Data analysis. In order to allow future comparison with genetic diversity after all silvicultural techniques (logging, grazing, and burning) are implemented, the data were analyzed in terms of eight plots, which represent the half plots subsequently burned (Table 3).

For *P. ponderosa* and *P. tridentata,* results were analyzed using Popgene version 1.21 (Yeh et al. 1997). A locus was considered polymorphic if an alternate allele occurred at least once. Statistics calculated included unbiased genetic distances (Nei 1978), expected heterozygosity (Nei 1973), inferred gene flow ($Nm = 0.25(1/F_{st})/F_{st}$; Slatkin and Barton 1989), and F statistics ($F = (H_e - H_o)/H_e$; Hartl and Clark 1989). The gene diversity statistics H_{es} (expected heterozygosity at the species level) and H_{ep} (expected heteozygosity at the population level) were calculated (Hamrick and Godt 1990).

For *F. idahoensis,* phenotypic diversity measures were calculated from both band presence/absence and multi-band patterns. For presence/absence data, phenotypic diversity was measured by a polymorphic index (PI), based on the frequency of occurrence of each band. PI = the sum of $f(1-f)$, where f = the frequency of a band in a population (Chung et al. 1991). For multi-band patterns, phenotypic diversity measures include: (1) the number of bands found in each plot, (2) percent of stains that yield more than one band pattern, (3) the average number of band patterns per stain in each plot, and (4) Shannon-Weaver Diversity Index values (Shannon and Weaver 1949). The Shannon-Weaver Diversity Index uses the frequency of each band pattern in each plot. The larger the Shannon-Weaver Index,

TABLE 3. SUMMARY OF GENETIC DIVERSITY MEASURES IN *PINUS PONDEROSA* AND *PURSHIA TRIDENTATA* BY SPECIES AND BY PLOT. All = overall statistics for the study. Mean = average over the 8 plots. SD = standard deviation. N = mean number of individuals sampled per locus, per population. %P = percent of all loci that are polymorphic. A = average number of alleles per locus. A_p = the average number of alleles per polymorphic locus. H_o = observed frequency of heterozygotes. H_e = frequency of heterozygotes expected under Hardy-Weinberg equilibrium conditions. F = the fixation index, = $(H_e-H_o)/H_e$ (* = statistically significant difference ($p < 0.05$)). S-W = Shannon-Weaver diversity index. B and N following plot numbers indicated the half plots that were subsequently burned or not burned.

	N	%P	A	A_p	H_o	H_e	F	S-W
Pinus ponderosa (26 loci)								
All	207	92%	3.73	3.96	0.264	0.272	0.030	0.510
Mean	26	85%	2.79	3.09	0.265	0.271	0.021	0.483
SD	2.4	4.0	0.11	0.10	0.021	0.010	0.082	0.019
38B	24	88%	2.73	2.96	0.263	0.269	0.023	0.477
38N	26	81%	2.73	3.14	0.251	0.264	0.046	0.470
39B	27	92%	3.04	3.21	0.283	0.287	0.012	0.517
39N	29	85%	2.81	3.14	0.240	0.277	0.133	0.495
41B	26	85%	2.73	3.04	0.280	0.280	0.002	0.495
41N	23	81%	2.77	3.19	0.257	0.255	−0.010	0.455
43B	23	88%	2.73	2.96	0.301	0.264	−0.140	0.467
43N	29	81%	2.77	3.09	0.244	0.272	0.104	0.489
Purshia tridentata (16 loci)								
All	404	75%	2.38	2.83	0.138	0.157	0.117*	0.263
Mean	50	55%	1.81	2.47	1.138	0.156	0.113	0.254
SD	8.0	9.6	0.12	0.26	0.006	0.005	0.049	0.011
38B	50	50%	1.69	2.38	0.135	0.148	0.087	0.235
38N	50	56%	1.69	2.22	0.134	0.152	0.120*	0.247
39B	62	38%	1.75	3.00	0.140	0.156	0.101	0.254
39N	38	50%	1.75	2.50	0.138	0.152	0.091	0.246
41B	52	69%	1.94	2.36	0.130	0.155	0.165	0.260
41N	50	62%	2.00	2.60	0.147	0.157	0.059	0.262
43B	42	52%	1.75	2.20	0.132	0.166	0.204*	0.269
43N	60	62%	1.94	2.50	0.147	0.159	0.076	0.261

the more diverse the plot. The distribution of the total variation within and among plots was determined by partitioning the total Shannon-Weaver Diversity Index. The phenotypic relationships among plots were determined by calculating Hedrick's phenotypic identities (Hedrick 1971) for multi-band pattern data, and by cluster and principle coordinate analyses of Jaccard's Similarity Index for band presence/absence data (Chung et al. 1991; Rolf 1987).

The hypothesis that all the plots had equal genetic diversity by species was tested by ANOVA using Excel (Microsoft 1997). In one analysis, plots were treated as blocks and the burned and unburned half plots as samples. In a second analysis, burned and unburned half plots were treated as blocks and the plots were treated as samples.

RESULTS

Pinus ponderosa, P. tridentata, and *F. idahoensis* were all genetically variable (Tables 3 and 4). Both percent polymorphic loci/enzyme and the Shannon-Weaver diversity index indicate that *P. tridentata* is the least variable of the three species.

In the two diploid species for which they could be calculated, observed heterozygosity nearly equaled that expected under Hardy-Weinberg conditions. Therefore, the fixation index (F) within each plot, calculated for *P. ponderosa* and *P. tridenta,* was low ($F < 0.113$) (Table 3). Although heterozygosity could not be calculated for *F. idahoensis,* we observed the unequal band staining and complex band patterns characteristic of heterozygous tetraploids (Soltis and Riesberg 1986).

F_{st} values indicated that over 98% of the isozyme variation in *P. ponderosa* and *P. tridentata* was within, rather than between populations, and inferred gene flow was high (Table 5). G_s, a measure of interpopulation diversity analogous to F_{st} but calculated from the Shannon-Weaver diversity index, was somewhat higher than the corresponding values of F_{st}, but indicated that in all three species more than 92% of the variation was within, rather than between, populations (Table 5).

Genetic similarities among plots were correspondingly high. Unbiased genetic identities (Nei 1978) between plots were greater than 0.99 for *P. ponderosa* and *P. tridentata.* Hedrick's distances (Hedrick 1971), calculated using band patterns, revealed a similarily greater than 0.98 for *F. idahoensis.* For *F. idahoensis,* band presence/absence data did not reveal differences between plots. No bands were unique to any plot. Two clusters appeared in a graph based on Jaccard's similarity index (not shown), but all eight plots were represented in both clusters.

TABLE 4. GENETIC VARIATION IN *FESTUCA IDAHOENSIS* AT BLACK'S MOUNTAIN, FOR 12 ENZYMES. All = overall statistics for the study. Mean = average over the 8 plots. SD = standard deviation. N = mean sample size/stain. #Bands = total number of bands (in all stains), in the population. %P* = percent of all presumed loci (regions on the gel that each probably represent a locus or set of homoeologos loci) that have more than one band pattern. A* = mean number of band patterns/stain. PI = polymorphic index based on band presence/absence data (see text). S-W = Shannon-Weaver diversity index is based on band patterns. B and N following plot numbers indicated the half plots that were subsequently burned or not burned.

	N	#Bands	%P*	A*	PI	S-W
All	385	53	83%	5.17	3.804	0.563
Mean	48.1	45.1	66%	3.41	3.666	0.521
SD	8.6	2.5	8.3%	0.24	0.211	0.015
38B	61	46	75%	3.83	3.400	0.535
38N	47	45	67%	3.33	3.639	0.498
39B	59	45	67%	3.58	3.851	0.543
39N	36	42	58%	3.08	3.840	0.511
41B	49	43	50%	3.17	3.536	0.527
41N	47	49	75%	3.58	3.530	0.525
43B	39	48	67%	3.33	4.013	0.516
43N	47	43	67%	3.42	3.520	0.510

With one exception, measures of genetic variability in plots 38, 39, 41, and 43 did not differ significantly for any of the three species. The only exception was the Shannon-Weaver diversity index for *P. tridentata.* Its Shannon-Weaver diversity index values differed significantly among plots (Table 6), and values for plot 43 are higher than those of plot 38. Before fire treatments were applied, measures of genetic variability were the same in all the half plots, except that the percent polymorphic loci and observed heterozygosity for *P. ponderosa* were consistently higher on the plots that would later be burned (Table 6).

The mean heterozygosity for the species (H_{es}) and the sampled populations (H_{ep}) (Hamrick and Godt 1990) were 0.271 and 0.266, respectively, for *P. ponderosa,* and 0.156 and 0.120 for *P. tridentata.*

DISCUSSION

Pinus ponderosa. Genetic variability found in this study is higher than previously reported in comparable studies of this species (that is, in studies that involved at least twelve loci and including both polymorphic and monomorphic loci) (Allendorf et al. 1982; Niebling and Conkle 1989; O'Malley et al. 1979; Woods et al. 1983; Yow et al. 1992). Expected heterozygosity (H_{es}) in this study equals 0.272 while the average H_{es} of the other studies equals 0.171. However, this level of genetic variability is consistent with previous NFGEL research on *P. ponderosa* (in previous NFGEL studies the average H_{es} = 0.231; NFGEL, unpubl.). The higher genetic variability reported by NFGEL for this species probably results from quality control measures and highly standardized procedures that allow repeatable detection of small differences in enzyme migration distances. Including rare alleles (those with frequencies lower than 0.05) in analyses may also contribute to the high genetic variability reported. Both NFGEL studies and previously published work indicate that *P. ponderosa* subsp. *ponderosa* is more genetically variable than *P. ponderosa* subsp. *scopulorum* (average from NFGEL studies: H_{es} (subsp. *ponderosa*) = 0.247, H_{es} (subsp. *scopulorum*) = 0.235; average from other studies: H_{es} (subsp. *ponderosa*) = 0.161, H_{es} (subsp. *scopulorum*) = 0.151).

Genetic variation in *P. ponderosa* was distributed within, rather than among, the plots. More than 90% of the isozyme variation often occurs within, rather than among, *P. ponderosa* populations (Hamrick et al. 1989, Linhart et al. 1981). Because *P. ponderosa* pollen can travel long distances (Latta et al. 1998), and calculated gene flow among plots in this study is high (Table 5), the short distances (2 to 4 km) between plots may limit genetic differentiation. However, genetic differentiation has been detected previously over small distances in *P. ponderosa* (Beckman and Mitton 1984; Mitton et al. 1977; Mitton et al. 1980). In general, the genetic similarily among plots provides a uniform background against which the genetic effects, if any, of timber management practices will be detectable. However, the higher initial percent polymorphic

TABLE 5. INTER-POPULATION DIVERSITY STATISTICS IN *PINUS PONDEROSA*, *PURSHIA TRIDENTATA* AND *FESTUCA IDAHOENSIS* AT BLACK'S MOUNTAIN. G_s = a measure of inter-populational genetic differentiation derived from the Shannon-Weaver diversity index and analogous to F_{st}. F_{st} = Wright's fixation index (Weir 1990). S-W = mean Shannon-Weaver diversity index. Nm = inferred gene flow, = $0.25(1-F_{st})/F_{st}$.

Species	G_s	F_{st}	Nm
Pinus ponderosa	0.0406	0.0188	13.0301
Purshia tridentata	0.0708	0.0148	16.6675
Festuca idahoensis	0.0637		

TABLE 6. ANALYSIS OF VARIANCE OF MEASURES OF GENETIC VARIABILITY IN *PINUS PONDEROSA*, *PURSHIA TRIDENTATA* AND *FESTUCA IDAHOENSIS*. The fixation index $F = (H_e-H_o)/H_e$. The variance ratio $F = s_1^2/s_2^2$, where s^2 = the variance of the sample. * = statistically significant difference ($p < 0.05$).

Measure of genetic variance	Half plots (half later burned)		Plots 38, 39, 41 and 43	
	F (variance ratio)	probability	F (variance ratio)	probability
Pinus ponderosa				
N = number of individuals/plot	1.0576	0.3434	0.7233	0.5886
P = % polymorphic loci	12.755	0.0118*	0.5460	0.6767
A = alleles/locus	0.2242	0.6526	2.3785	0.2107
A_p = alleles/polymorphic locus	2.4440	0.1690	0.9505	0.4964
H_o = observed heterozygosity	14.627	0.0087*	0.1305	0.9370
H_e = expected heterozygosity	1.2605	0.3045	1.0604	0.4588
F = fixation index	3.5968	0.1067	0.3551	0.7893
SW = Shannon Weaver diversity index	0.6778	0.4418	1.4773	0.3478
Purshia tridentata				
N = number of individuals/plot	0.1076	0.7540	0.0059	0.9993
P = % polymorphic loci	0.5627	0.4815	3.8774	0.1117
A = alleles/locus	0.4615	0.5223	6.0143	0.0579
A_p = alleles/polymorphic locus	0.0238	0.8824	1.5350	0.3355
H_o = observed heterozygosity	3.2858	0.1198	0.1758	0.9076
H_e = expected heterozygosity	0.1197	0.7412	4.9700	0.0777
F = fixation index	2.9523	0.1366	0.2069	0.8868
SW = Shannon Weaver diversity index	0.0031	0.9574	6.6854	0.0489*
Festuca idahoensis				
N = number of individuals/plot	1.8075	0.2274	0.4115	0.7542
Number of bands/enzyme	0.1617	0.7015	0.2770	0.8401
%P* = % polymorphic enzyme	0.1005	0.7619	0.3481	0.7938
A* = number of patterns/enzyme	0.4906	0.5099	0.2970	0.8269
PI = polymorphic index	0.1811	0.6853	1.4484	0.3542
SW = Shannon Weaver diversity index	5.6936	0.0543	0.3223	0.8104

loci and observed heterozygosity in burned than in unburned half-plots (Table 6) would have been considered a treatment effect if this baseline study had not been done.

Purshia tridentata. Bitterbrush has been the subject of intense scrutiny, focused on interspecific relationships and management practices (e.g., Basile 1967) rather than genetic diversity. Secondary compounds have interferred with isozyme resolution in previous studies (S. Brunsfeld, pers. comm.). Genetic variation was less evenly distributed among plots in *P. tridentata* than in the other two species in this study (Table 3), possibly because *P. tridentata* is pollinated by insects, rather than wind. Although plots were homogeneous for most measures, there were significant differences in the fixation index (Table 3) and Shannon-Weaver diversity index (Table 6).

Festuca idahoensis. Because polyploidy complicates gel interpretation, isozymes have been underutilized for describing genetic variation in the fine-leaved fescues, *Festuca* subgenus *Festuca,* to which *F. idahoensis* belongs, and summary statistics are rarely reported. One should keep certain trends in mind when comparing a phenotypic analysis, like that performed for fescues, with genotypic analyses of isozymes. The proportion of polymorphic enzymes (%PE in Tables 7, 8) is higher than the percent polymorphic loci (%P*) because stains may reveal both polymorphic and monomorphic loci for the same enzyme. %PE is reported because the statistic is unambiguous. Percent polymorphic putative loci (%P*) is theoretically equal to %P of a genetic analysis, although for polyploid plants different researchers may parse band patterns into loci in different ways. The number of patterns reported per putative locus (A*) should be somewhat greater than the number of alleles per locus, because any two alleles can produce three patterns. For example, AA homozygotes, BB homozygotes, and AB heterozygotes are counted as three different patterns. The polymorphic index (PI) is a rough measure of heterozygosity, valid only for comparing populations within a study. Measures of similarity and diversity based on patterns (Hedrick's distance and the Shannon-Weaver diversity index, respectively) may overestimate differences, because AA homozygotes, BB homozygotes, and AB heterozygotes are considered three equally different patterns. On the other hand, measures of similarity and diversity based on bands (Jaccard's Similarity Index and the polymorphic index, respectively) produce uneven results among monomeric enzymes (which have two bands when heterozygous), dimers (which have three), and tetramers (which have five) (Gottlieb 1977). The Gs statistic derived from partitioning the Shannon-Weaver diversity index, like hierarchical F-statistics (Wright 1978), indicates whether a greater proportion of variation resides within

TABLE 7. OVERALL ISOZYME DIVERSITY STATISTICS FOR TAXA *FESTUCA* SUBGENUS *FESTUCA*, FROM STUDIES THAT WERE NOT LIMITED TO POLYMORPHIC ALLELES. Overall statistics are total values for the entire study. Chr. = Chromosome number (2X = 14, etc.). † = chromosome numbers from Markgraf-Dannenberg (1980); other chromosome numbers were provided in the source article. Pops. = number of populations. N = mean sample size per population. Enz = number of enzymes stained. Loci = number of regions on the gel that each probably represent a locus or set of homoelogous loci. Summary statistics calculated by authors: %PE = percent of polymorphic enzymes. %P = percent of polymorphic putative loci. AE = number of patterns per enzyme. A* = number of patterns per putative locus.

Taxon	Chr.	Pops.	N	Enz.	Loci	%PE	%P*	AE	A*	Source
(*F. ovina* complex)										
F. auriculata	2X	4	20	10	15	90%	67%			1
F. baffensis	4X	3	26	10	15	90%	67%			1
F. brachyphylla	6X	5	23	10	15	80%	67%			1
F. brevissima	2X	2	23	10	15	20%	13%			1
F. idahoensis	4X	8	48	11	12	82%	83%		5.17	5
F. idahoensis	4X	8	41	11	18	91%	67%		2.67	4
F. minutilfora	2X	1	3	9	14	67%	21%			1
F. roemeri v. *klamathensis*	4X	4	31	11	18	91%	67%		2.78	4
F. roemeri v. *romeri*	4X	8	27	11	18	91%	72%		4.00	4
F. valesiaca	2X	3	31	8	20	75%	50%	2.75	1.65	2
(*F. rubra* complex)										
F. amythestina	2X†	2	58	8	20	62%	35%	2.25	1.40	2
F. asperifolia		1	40	8	20	75%	45%	2.25	1.65	2
F. diffusa	6X, 8X†	1	40	8	20	50%	20%	1.65	1.35	2
F. heterophylla	4X†	8	37	8	20	65%	40%	2.25	1.60	2
F. nigrescens	4X, 6X†	6	36	8	20	50%	35%	2.50	1.65	2
F. nigrescens	6X	3	27	11	18	82%	67%		2.33	4
F. peristerea		1	40	8	20	75%	30%	1.75	1.35	2
F. picturata		3	34	8	20	75%	35%	2.12	1.50	2
F. rubra	2X-10X†	8	20	8	20	75%	45%	2.88	1.75	2
F. rubra	6X	6	38	10		100%				3

Sources:
1 = Aiken et al. 1993
2 = Angelov and Edreva 1987 (omitting anodal esterase), Angelov et al. 1988, Angelov 1992, 1993
3 = Livesey and Norrington-Davies 1991
4 = Wilson 1999
5 = this study

or among populations. Although a phenotypic analysis is imprecise compared to genetic analyses of isozyme data, it does quantify isozyme variation and therefore allows comparisons among those polyploid taxa for which genetic interpretations are not possible.

Available studies indicate that these fescues are highly polymorphic (Tables 7, 8), with the excep-

TABLE 8. MEAN ISOZYME DIVERSITY STATISTICS PER POPULATION FOR *FESTUCA* SUBGENUS *FESTUCA*, FROM STUDIES THAT WERE NOT LIMITED TO POLYMORPHIC ALLELES. Chr. = Chromosomes (2X = 14, etc.). Chromosome numbers were provided in the source article. Pops. = number of populations. N = mean sample size per population. Enz = number of enzymes stained. Loci = number of regions on the gel that each probably represent a locus or set of homoeologos loci. Summary statistics calculated by authors: %PE = percent of polymorphic enzymes. %P* = percent of polymorphic putative loci. A* = number of patterns per putative locus. S-W = Shannon-Weaver diversity index.

Taxon	Chr.	Pops.	N	Enz.	Loci	%PE	%P*	AE	A*	Source
(*F. ovina* complex)										
F. auriculata	2X	4	20	10	15	60%	43%			1
F. brachyphylla	6X	5	23	10	15	67%	40%			1
F. brevissima	2X	2	23	10	15	68%	7%			1
F. idahoensis	4X	8	48	11	12	68%	66%	3.41		3
F. idahoensis	4X	2	56	11	18	68%	50%	2.03	0.2032	2
F. minutilfora	2X	1	3	9	14	67%	21%			1
F. roemeri v. *klamathensis*	4X	4	31	11	18	80%	53%	1.89	0.2376	2
F. roemeri v. *romeri*	4X	6	33	11	18	67%	50%	1.94	0.2710	2

1 = Aiken et al. 1993
2 = Wilson 1999
3 = this study

tion of the uncommon *F. brevissima* Jurtzev. Variability in Black's Mountain *F. idahoensis* was high but consistent with previously reported fescue genetic variation. Our reported number of band patterns per putative locus was particularly high but not anomalous. For example, we detected 17 PGI band patterns (Shannon-Weaver diversity index = 1.91), and the same number were found for PGI in a survey of 6 European Red Fescue (*F. rubra* L.) populations (S-W = 1.75) (Livesey and Norrington-Davies 1991). That study reported an average of 10 band patterns per enzyme in the three highly variable enzymes investigated.

Genetic distances and identities among fescue populations are rarely reported. Genetic identities between populations of diploid arctic fescues are 0.934 (*F. brevissima*) and an average of 0.857 (*F. auriculata* Drobov aggregate) (Aiken et al. 1993). In both intra- and interspecific comparisons, Hedrick's identities exceed 0.95 between populations in the tetraploid *F. idahoensis* and Roemer's Fescue (*F. roemeri* (Pavlick) E. B. Alexeev) species pair in northern California (Wilson 1999). The high (greater than 0.98) Hedrick's identities among Black's Mountain fescue populations are therefore expected.

Because Black's Mountain populations of *F. idahoensis* were similar, any effects of burning, grazing, and logging regimes on genetic variability will be detectable. Such fine-scale adaptation to local habitat variables has been seen in grasses, particularly in self-pollinating species (Bradshaw 1959; Clary 1975; Clegg and Allard 1972; Hamrick and Allard 1972; Kahler et al. 1980; Lönn 1993; Nevo et al. 1983). However, coarse adaptation has also been observed in both self- and cross-pollinated introduced grasses (Rice and Mack 1991; Rapson and Wilson 1988).

Summary. The gymnosperm tree *P. ponderosa*, the dicot shrub *P. tridentata,* and the monocot grass *F. idahoensis* are not phylogenetically close. However, they have strikingly similar patterns of electrophoretically detected genetic variation. All are genetically variable, with well over 90% of the variation within, rather than among, populations in the area studied. All three are common, widespread, long-lived, perennial, outcrossing species that dominate late successional stages in their plant community. Plants with this series of characteristics tend to be more genetically variable than average, and to have their genetic variation within, rather than among, populations (Hamrick and Godt 1990). The high level of genetic variability detected in the three plants is consistent with observed trends in genetic variability (Hamrick and Godt 1990).

The markedly lower genetic variation in *P. tridentata* as compared to the other two species is consistent with the tendency for insect pollinated plants and dicots to have much less isozyme variation than wind pollinated plants and gymnosperms or monocots (Hamrick and Godt 1990).

The value of the genetic diversity statistics H_{es} and H_{ep} for *P. ponderosa* are more than one standard deviation higher than comparable mean statistics reported (Hamrick and Godt 1990; Hamrick et al. 1992). Those for *P. tridentata* vary from that far above to somewhat below average, depending upon the comparison; they are low for woody angiosperms (Hamrick et al. 1992), but most woody angiosperms studied are wind-pollinated trees, and *P. tridentata* is an insect-pollinated shrub. For all three species, the percentage of genetic variability among populations is lower than the mean reported in comparable plants (Hamrick and Godt 1990), but few other studies compared populations growing in such close proximity in the same habitat.

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